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AN EVALUATION OF CLADOCERA AS A BIOASSAY ORGANISM.(U)

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AN EVALUATION OF CLADOCERA AS A BIOASSAY ORGANISM

JUNE 1976

Final Report: October 1975 to February 1976

Approved for public release; distribution unlimited.

AIR FORCE CIVIL ENGINEERING CENTER
(AIR FORCE SYSTEMS COMMAND)
TYNDALL AIR FORCE BASE
FLORIDA 32403

branchiopod crust water fleas

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The literature review of Cladocera, with special emphasis on Daphnia, was conducted to assess their use in aquatic pollution research and new methods to measure the effects of pollution on the aquatic ecosystem. A discussion on life cycle, metabolism cyclomorphosis, filtering rate, culture techniques, toxicity, and taxonomy is included.

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PREFACE

This report documents work performed during the period 1 October 1975 through 4 February 1976 at the OL-AA Air Force Civil Engineering Center, Air Force Systems Command, Kirtland Air Force Base, New Mexico 07117.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

CLAUDE L. SCOTT, Sgt, USAF
Project Officer
Environmental Planning Div

RUTHERFORD C. WOOTEN
Maj, USAF, BSC
Chief, Envmtl Planning Div

DONALD G. SILVA, Lt Col, USAF, BSC
Director of Environics

ROBERT E. BRANDON
Technical Director

ROBERT M. ITEN, Colonel, USAF
Commander

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SECTION I

INTRODUCTION

PURPOSE AND SCOPE

This literature review was conducted to assess the use of the Cladocera as a bioassay organism and those aspects of its biology that might influence such experimental work. The literature review is centered around the most common genera, Daphnia (Figure 1), due to consideration for using it as a bioassay organism in Air Force laboratories, and agencies involved in environmental quality research.

BACKGROUND

Historically, Daphnia has been used as a bioassay organism in a large number of toxicity studies. Over a hundred years ago Paul Bert, as reported by Anderson (Reference 1), used daphnids in a report on the toxicity of salt solutions. He also indicated that during the first half of the century, Anderson, Naumann, and other researchers published papers on the use of Daphnia as an experimental organism in toxicity studies.

There are many reasons why Daphnia is so widely used in toxicity studies:

1. They reproduce by diploid parthenogenesis, and production can be limited to females which are genetically identical animals, discounting mutations.

2. They are easily cultured in large or small containers, and can be fed a number of foods all with satisfactory results.

3. A large number of known age animals can be available at any time.

4. They are sensitive to a large number of toxic materials.

5. Their small size.

GENERAL CHARACTERISTICS

Daphnia, commonly called "water fleas" (Figure 1), are members of the class Crustacea, order Cladocera, and are chiefly freshwater organisms, with few marine representatives. They

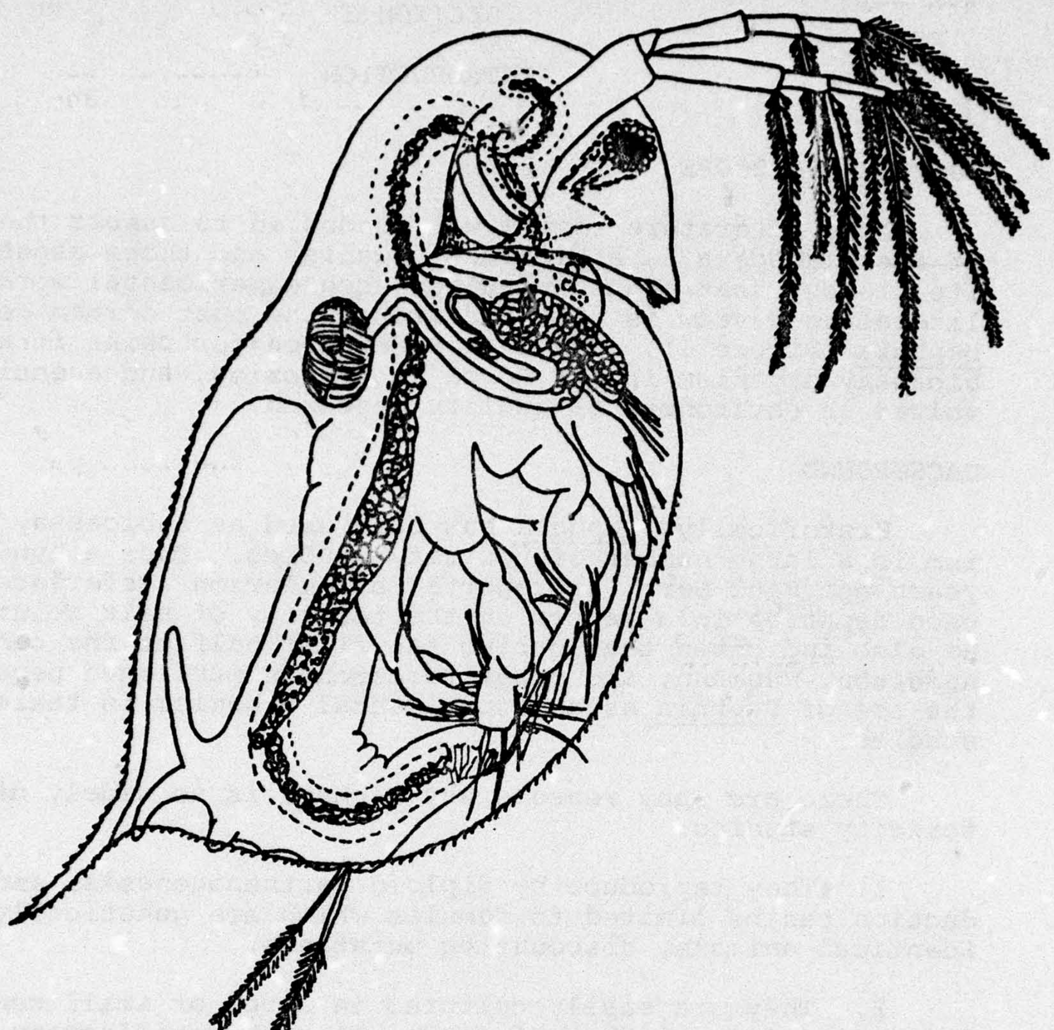

Figure 1. Anatomy of female Daphnia pulex (Taken from Pennak, Reference 2).

are small, varying from 0.2 mm to about 5.0 mm in length. Limnetic species are usually light-colored and translucent. Pond, littoral, and bottom species are darker in color. Daphnia is an important source of food for small and large fish and immature and mature aquatic insects.

Daphnia move by a series of "hops" produced by more rapid and vigorous strokes. Some Cladocera including Holopedium and Scapholeberis swim upside down. Daphnia are weak swimmers and do not inhabit swift streams or brooks.

Daphnia are filter feeders. This is accomplished by movement of the highly setose thoracic legs which produce a constant current between the valves. These movements filter food particles from the water and collect them in a median ventral groove at the base of the legs. The food particles are then ground between the mandibles and taken into the mouth (Reference 2).

Algae and protozoa are their chief foods, but indications are that bacteria and detritus make up an important part of their diet (Reference 3). It was previously thought that Daphnia simply ingested all material they filtered from the water without any selective mechanism. However, several reports have indicated that Daphnia are capable of selective feeding (References 4, 5, 6).

Reproduction in Daphnia is parthenogenetic during the greater part of the year. Usually one clutch of eggs is released into the brood chamber during each adult instar. Depending on the species and environmental condition, the number of eggs per clutch varies. MacArthur and Baillie (Reference 7) found that the average total young production for individual females raised at 28°C was 15, at 19°C was 49, and at 8°C was 36. Anderson and Jenkins (Reference 8) record production of three or four times as many for the same temperatures.

Reproduction is usually parthenogenetic; resulting only in female offspring; however, production of males may be induced by crowding, decrease in food concentration, and water temperature. Under these conditions, some parthenogenetic male eggs are released into the brood chamber. If these conditions persist sexual eggs will appear. The females which produce these sexual eggs are morphologically similar to the parthenogenetic females, but are capable of copulation with males. The females produce one, two, or sometimes several sexual "resting" eggs. These eggs have a

wall which becomes thickened and darkened to form an ephippium. In Daphnia the ephippium separates from the rest of the shell at the subsequent molt. The ephippium, upon release, either sinks to the bottom or floats upon the surface. The ephippia and the eggs inside are able to withstand freezing and drying. This enables Daphnia to inhabit ponds which are dry for extended periods of time.

SECTION II

REVIEW OF LITERATURE

LIFE CYCLE

The life cycle of the Cladocera is of the order of days and there are no free egg or larvae stages in the life cycle. Some males are produced but in such small numbers that sex ratios can be ignored. Except for possible mutation or position-effects due to crossing over, the offspring of a single female are genetically identical. In effect, a clonal population can be maintained so that the possible interactions between population dynamics and genetics can be ignored.

Pratt (Reference 9) has shown, however, that Daphnia magna populations will oscillate in number without any apparent environmental change. Since the 17th Century, it has been known that populations of Cladocera in nature show pronounced maxima. Because an infinite number of environmental factors could be postulated as explanations for these maxima, it would be possible to consider these maxima as environmentally determined (Reference 10).

The life span from release of the egg into the brood chamber until the death of the adult is highly variable depending on the species and environmental conditions. In laboratory cultures, Daphnia longispina usually lives for 28 to 33 days. Daphnia magna as found by MacArthur and Baillie (Reference 7) lived an average of 26 days at 28°C, 42 days at 18°C, and 108 days at 8°C. Length of life may also be increased by poor food supply. Limnetic individuals undoubtedly live through the entire winter at low temperatures.

Pennak (Reference 2) recognized four distinct periods in the life history of a cladoceran: egg, juvenile, adolescent, and adult. Segmentation begins promptly after a clutch of eggs is released into the brood chamber. The young, in the first juvenile instar, are similar in form to the adult, and are released from the brood chamber in about two days. Although greatest growth occurs during these stages, there are but few juvenile instars. Moina macrocopa has two juvenile instars, Daphnia longispina has three, Daphnia pulex has three or four and Daphnia magna three to five. The adolescent period is a single instar between the last juvenile instar and the first adult instar; during this instar the first clutch of eggs reaches full development in the ovary. As soon as the animal molts at the end of the adolescent instar and enters the first

adult instar, the first clutch of parthenogenetic eggs is released into the brood chamber. The second clutch of eggs is developing in the ovary during the first adult instar. Successive adult instars and new clutch of young are produced in a similar manner. However, there is often a sterile period during the last few instars of life.

The number of adult instars is much more variable than the number of juvenile instars. Daphnia pulex usually has 18 to 25 adult instars, Daphnia longispina 10 to 19, and Daphnia magna 6 to 22.

Growth, in terms of increase in size, as in all other Crustacea becomes apparent only immediately after each molt. During juvenile instars there may be almost a doubling of size after each molt. The increase in volume occurs within a few seconds or minutes and before the new exoskeleton hardens and loses its elasticity.

As already inferred, the duration of a single adult instar is highly variable, from a day to several weeks, although about two days under favorable conditions. Four events follow one another in rapid succession, usually in a few minutes to a few hours at the close of each adult instar:

1. The release of young from the brood chamber to the outside.

2. Molting.

3. Increase in size.

4. The release of a new clutch of eggs to the brood chamber.

METABOLISM

Metabolism may be defined as the sum of the processes or chemical changes in an organism or a single cell by which food is built up into living protoplasm and by which protoplasm is broken down into simpler compounds with the exchange of energy (Reference 11).

All living organisms carry out synthetic reactions, growing, reproducing, and performing work. Even in those processes in which the final "energy product" does appear eventually as heat, the overall reaction involves many individual steps, some of which are energy-yielding and some of which require energy (Reference 12).

Studies indicate (References 13 and 14) that the male metabolic rate in *Daphnia magna* is higher than that of the female, and that in both sexes the longevity is inversely proportional to the metabolic rate. If exposed to a range of temperatures, the temperature coefficients differ markedly for the sexes. At all temperatures, the inverse ratio between length of life and metabolic rate is maintained.

All three temperatures (8°C, 18°C, 28°C) within the normal range as evidenced by behavior, growth, and reproduction closely approximate a constant figure for both sexes, as though average life terminates after an outlay of energy bearing a definite ratio to the constant total of heart beats (Reference 13). One may conclude here that the relative longevity of the sexes may be expressed as varying inversely with their average rate of metabolic activity. The average age at death for males was 37.8 days, and for females 43.53 days. The duration of life for females exceeded that for males by 13.2 percent.

The available evidence also indicates that length of life in *Daphnia magna* has the usual temperature coefficient for a chemical reaction. Other factors being equal, it would seem that longevity is a function of metabolic rate, at least with *Daphnia magna* (Reference 14).

CYCLOMORPHOSIS

Brooks (Reference 15) defines cyclomorphosis as cyclic form change in a series of genetically identical generations. It occurs in species of fresh-water dinoflagellates, rotifers and cladocerans. Cyclomorphosis or seasonal changes in morphology, especially in females of limnetic species such as *Daphnia pulex* and *Daphnia longispina*, is one of the most intriguing and puzzling of all cladoceran problems. All of these reproduce by asexual or parthenogenetic processes. Many species of *Daphnia* which inhabit the open portion of all but the smallest bodies of fresh-water exhibit striking seasonal form changes (Reference 15).

Pennak gives a good account of the cyclomorphid conditions that occurs in the Cladocerans. During the late fall, winter, and early spring, a population of a cyclomorphic species has a homogeneous "normal" or round-headed form. As the water becomes warmer and the population develops, however, there is commonly a progressive increase in the longitudinal axis produced by a general elongation of the head and the appearance of a "helmet" (Figure 2). Characteristically, the helmets become fully developed by midsummer, when they may be quite

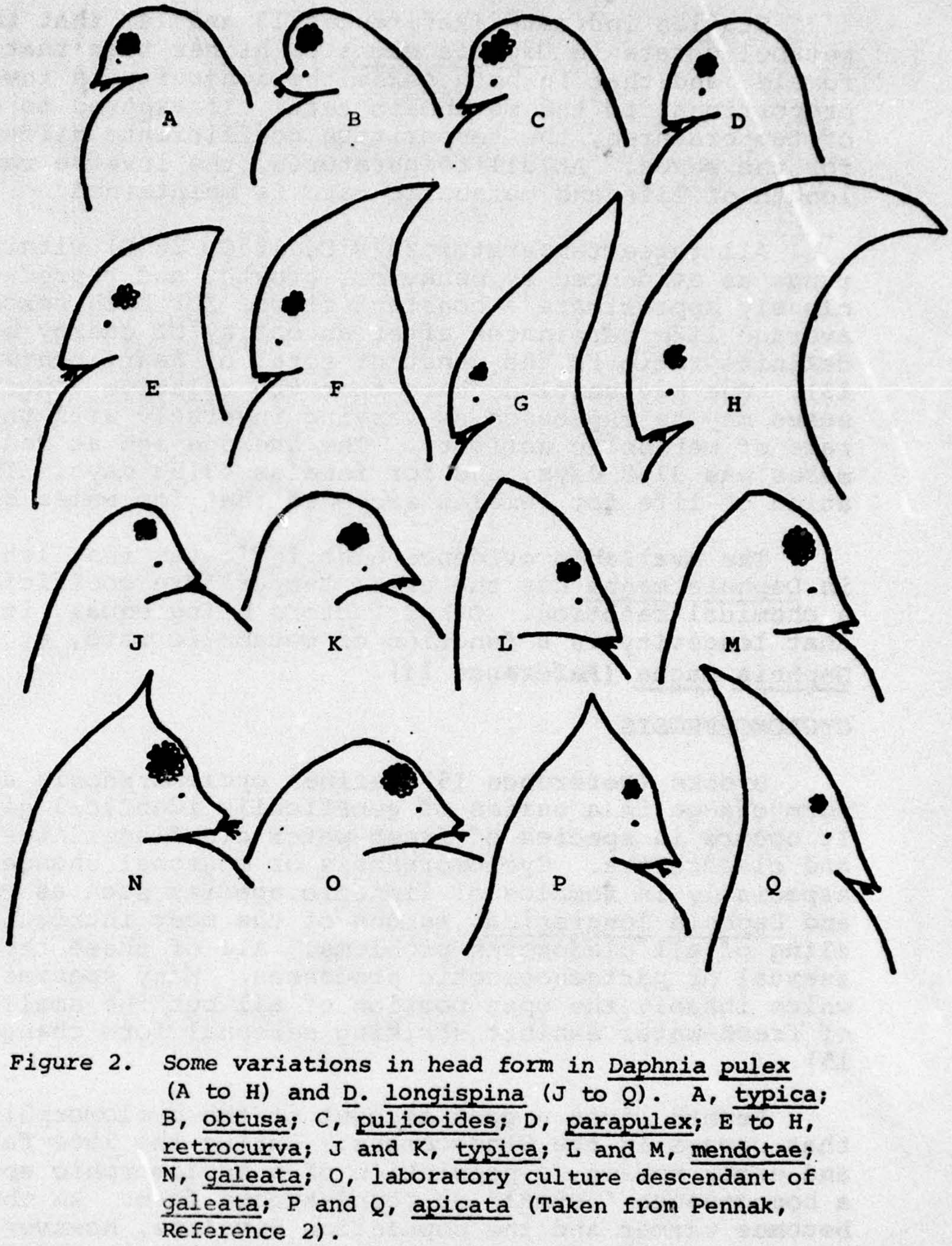

Figure 2. Some variations in head form in Daphnia pulex (A to H) and D. longispina (J to Q). A, typica; B, obtusa; C, pulicoides; D, parapulex; E to H, retrocurva; J and K, typica; L and M, mendotae; N, galeata; O, laboratory culture descendant of galeata; P and Q, apicata (Taken from Pennak, Reference 2).

bizarre (Figure 2). Beginning in the late summer or early autumn, the morphology of the head progressively reverts so that the "normal" head condition prevails by late autumn. Cyclomorphosis may also involve changes in size of eye and length of the posterion spine, in addition to the changes in the shape of the head.

The fact that the degree of summer helmet development differs widely in the same species, even in two neighboring lakes, presents a complicated problem. Cyclomorphosis is less pronounced in ponds and shallow lakes, and the degree of helmet development is relatively consistent from one individual to another, at any one time. However, in larger and deeper lakes cyclomorphosis and the degree of helmet development in a population are much more variable. Both strongly developed and poorly developed helmets, as well as integrades, may be found in the same townet sample.

Temperature appears to be a critical factor that influences cyclomorphosis, particularly during the early stages of development. Controlled experiments of Coker and Addlestone as reported by Pennak (Reference 2) shed some light on this factor. They found that the last one-third of the period of development of the first juvenile instar in the brood chamber was the critical period for temperature changes. Daphnia longispina raised at a temperature of 10°C or below during this period had round heads; 15 percent of those raised at 12°C had pointed heads; 33 percent of those raised at 13°C had pointed heads; and all of those raised at 16°C or more had pointed heads. In general, the higher temperatures produced more prominent helmets.

Banta (Reference 16) indicates that temperature is by no means the whole story and that genetic factors probably play an important role. He collected ten distinct morphological types of Daphnia pulex in the field and cultured them in the laboratory. Under such circumstances the pronounced helmets were lost or minimized, but each of the ten types remained recognizable and peculiar to itself.

FILTERING RATE

The importance of studies on filtering rate and other natural phenomenon associated with the feeding behavior of Daphnia cannot be overemphasized. Many studies have been done on the environmental conditions that affect feeding, assimilation, and respiration in Daphnia (Reference 17). These studies using radioactive algae have demonstrated the various mechanisms which regulate feeding (References 18, 19, 20). In 1969 Schindler used Daphnia magna, to test the effect of light, motion, crowding, animal weight and reproductive state upon assimilation, feeding, and respiration. In this study, light intensity, motion, and crowding did not cause significant changes in assimilation, feeding, and respiration rates. Reproductive state, animal weight, diet, and food concentration, however, did cause significant changes in one or more of these life processes (Table 1).

Table 1. THE EFFECTS OF ENVIRONMENTAL FACTORS ON ASSIMILATION, FEEDING, AND RESPIRATION RATES OF DAPHNIA MAGNA

Taken from Schindler 1968 (Reference 17)

Factors	Effects on: Assimilation	Feeding	Respiration
Light intensity (1000 ft candles versus dark)	NS	NS	NS
Motion (rotation at 1 rpm versus motionless)	NS	NS	NS
Crowding (one Daphnia/2 ml versus one Daphnia/10 ml)	NS	NS	NS
Reproductive state (ovigerous versus non-ovigerous)	S	NS	NS
Diet species (Chlorella, Chlamydomonas, Anabaena)	S	NS	Not tested
Food concentration (1-10 mg/l)	S	S	Not tested
Food energy content (2-5 cal/mg)	S	S	Not tested
Animal weight (of animals 1-4 mm in length)	S	NS	S
Water temperature (10 versus 20°C)	S	NS	S

NS = Not significant
S = Significant

For Daphnia and other filter feeders, three possible mechanisms of selection exist:

1. Mechanical selection, based on physical attributes of the food particles.

2. Chemical solution, based on substances present in the cell, or released by the cell.

3. Behavioral selection, based on different behavior patterns of the Daphnia species (Reference 21).

These mechanisms will vary from species to species. For example, Daphnia magna, the largest of the Daphnia could ingest particles that Daphnia galeate could not. The age of the individual would also influence the size of particles ingested. The type of substance released by the cell would have varied effects. The degree of effect would directly relate to age, sex, and species of Daphnia.

The feeding rate of Daphnia is proportional to the concentration of food below critical concentration. After a critical concentration is reached, the feeding rate decreases. McMahon and Rigler (Reference 22) observed that upon exposure of Daphnia magna to 10^6 cells/ml (which is above critical concentration), frequency of movement of the thoracic appendages slowly decreased and rejection of food began 15-20 minutes after exposure.

In nature it has been observed that Daphnia are often scarce in water rich with phytoplankton. Harvey (Reference 23) provided the "grazing" theory. The theory states that due to the effect of grazing by large populations of zooplankton, such as Daphnia, high concentrations of phytoplankton can only develop in areas where the population of zooplankton is relatively small or absent.

Hardy's theory of "animal exclusion" (Reference 24) is another possible explanation for this phenomenon. Hardy and Gunther (Reference 25) believed that large populations of phytoplankton have an inhibitory effect on feeding rate of zooplankton. It has been shown that many species of blue-green algae are either toxic to Daphnia or do not provide sufficient nutrition to support a population (Reference 26).

CULTURE TECHNIQUE

A suitable culture technique to culture Daphnia for bioassay should meet several criteria.

1. It should maintain the culture through several generations.

2. Produce only parthenogenetic females.

3. Supply a large number of known aged individuals with a minimum of time, space, and expense (Reference 27).

Table 2 describes a number of different culture techniques. Although not all of these techniques were used to culture *Daphnia* for bioassay, it does indicate the wide range of methods used. All of these techniques apparently satisfy the first two criteria, but only one of the methods used by Dewey and Parker (Reference 27) meets all three.

TOXICITY

Daphnia magna is the most widely used species of *Daphnia* for toxicity studies. This organism has been used to evaluate the presence of laxatives, and in assaying snake venoms, marijuana, vitamin E, aphrodisiacs, and several irritants (References 28, 29). Anderson (References 30, 31) used *Daphnia magna* to test Lake Erie water for toxicity of DDT and chlorides of various metals. This species has also been used in toxicity studies of herbicides and pesticides (References 32, 33, 34, 35).

Daphnia and other Crustacea in general are more susceptible to toxic substances than are fish (Reference 28). Sanders (Reference 32) found that *Daphnia* were generally more sensitive to herbicides than seed shrimp, glass shrimp, sowbug, scud, or crayfish. The details of his results are given in Table 3. It has been shown that some herbicides may also cause long-term chronic effects by gradual reduction in numbers and changes in species composition (Reference 36).

It has been demonstrated that *Daphnia* are sensitive to a wide range of toxic material, but members of different clones may show physiological differences to these toxic materials. Sanders (Reference 34) found that males have a higher metabolic rate, and are more susceptible to poisons than females. Banta found that ecdysis is a critical period of life of *Daphnia* and that exposure periods should be long enough to provide sufficient time for all *Daphnia* to molt. Anderson found that *Daphnia magna* initially molts in 20 hours at 25°C. From this it is important that toxicity studies be performed on females that are less than 20 hours old.

Table 4 is not intended to be an evaluation of toxicity studies but simply to give some idea of the types of studies that have been conducted and their results.

Table 2. *DAPHNIA* CULTURE TECHNIQUE

Water	Food	Temperature	Rearing Chamber	Species	Reference
artesian	algae and yeast	72° to 74°F	1 gal polyethylene funnels	*Daphnia magna*	(27)
unchlorinated natural water	manure-soil medium supplemented with yeast	19°C± 1°C	4 oz glass jars	*Daphnia magna*	(37)
Lake Superior water	0.5 g power grass 10 g trout-fry granules	not stated	250 ml beakers	*Daphnia magna*	(38)
lake water	algae	15°C	not stated	*Daphnia pulex* *Daphnia galeata*	(39)
tap water	cultured in association with snails	18° to 20°C	cement water table 66 x 24 in	*Daphnia magna*	(18)
not stated	algae and yeast	20°C	10 liter pyrex jars	*Daphnia magna*	(22)
spring water	algae	15°, 20°, 25°C	not stated	*Daphnia pulex* *Daphnia galeata* *Daphnia schidleri* *Daphnia magna*	(39)
spring water	yeast	room temp	not stated	*Daphnia magna*	(40)
pond water	algae	14°C± 1°C	small glass jars	*Daphnia obtusa*	(10)
lake water	algae	20°C	4 liter battery jars	*Daphnia pulex*	(26)
pond water	egg yolk, yeast, and algae	21° to 23°C	aquaria	*Daphnia pulex*	(41)
filtered water	algae and bacteria	12°C	small jars	*Daphnia rosia*	(42)
pond water	yeast	18°C	3000 ml aquariums	*Daphnia magna*	(43)

Table 2 (Concluded)

Water	Food	Temperature	Rearing Chamber	Species	Reference
not stated	yeast and algae also nutrient broth to stimulate bacteria	not stated	10 gal aquariums	Daphnia magna	(19)
filtered water	green algae	20°C	aquarium	Daphnia pulex	(44)
aerated tap water	algae	20°C	tanks	Daphnia magna	(45)
tap water	manure-soil modified from Banta	25°C	20 to 25 cc vials	Daphnia magna	(46)
pond water	green algae	18°C and 25°C	50 cc wide mouth glass bottles	Daphnia magna	(9)
well water	yeast	21.1°C ± 0.5°C	1 gal wide mouth glass bottles	Daphnia magna	(47)

TABLE 3. TOXICITY OF SOME HERBICIDES TO SELECTED FRESHWATER CRUSTACEANS AND FISH

48-hr TL_{50} for given Species and Temperature (mg/l)[a]

Herbicide	Crustaceans: Water flea (Daphnia magna) 21°C	Seed Shrimp (Cypridopsis vidua) 21°C	Scud (Gammarus fasciatus) 15.5°C	Sowbug (Asellus brevicaudus) 15.5°C	Glass shrimp (Palaemonetes kadiakensis) 21°C	Crayfish (Orconectes Nails) 15.5°C	Fish: Bluegill (Lepomis macrochiues) 24°C
Dichlone	0.025	0.12	0.24	0.20	0.45	3.2	0.12
2, 4-D (PGBE)	0.10	0.32	2.6	2.2	2.7	>100.0	0.90
Silvex (PGBE)	0.18	0.20	1.0	0.50	3.2	>100.0	16.6
Trifluralin	0.56	0.25	1.8	2.0	1.2	50.0	0.019
Molinate	0.60	0.18	0.39	0.40	1.0	5.6	0.475
Simazine	1.0	3.2	>100.0	>100.0	>100.0	>100.0	>100.0
Vernolate	1.1	0.24	20.0	5.6	1.9	24.0	9.2
Silvex (BEE)	2.1	4.9	0.74	40.0	8.0	60.0	70.0
2, 4-d (dimethylamine salt)	4.0	8.0	>100.0	>100.0	>100.0	>100.0	>100.0
2, 4-D (BEE)	5.6	1.8	5.9	3.2	1.4	>100.0	1.1
Dichlobenil	10.0	7.8	18.0	34.0	9.0	22.0	20.0
Amitrol-T	30.0	32.0	>100.0	>100.0	>100.0	>100.0	>100.0
Diphenamid	56.0	50.0	>100.0	>100.0	58.0	>100.0	80.0
Dicamba	>100.0	>100.0	>100.0	>100.0	>100.0	>100.0	40.0
Fenac (Na salt)	>100.0	>100.0	>100.0	>100.0	>100.0	>100.0	19.0
2,4-D (acid)	>100.0	-	3.2	-	-	-	-

[a]Taken from Sanders (Reference 33)

Table 4. EXAMPLES OF TOXICITY STUDIES

Toxic Substance	Species of Daphnia	Type of Study	Results	Reference
Sodium Salts	Daphnia magna	Acute toxicity	IC_{50} given	(48)
Chlorides of Metals	Daphnia magna	Toxicity threshold of 25 cation added to Lake Erie water	Thresholds were determined sodium chloride least toxic sodium chloride most toxic	(31)
Laboratory-grade rubber stoppers	Daphnia magna	Acute toxicity	All animals dead in 24 hrs	(49)
16 Aquatic Herbicides	Daphnia magna	Acute toxicity of 16 aquatic herbicides	IC_{50} was determined	(47)
Pesticides	Daphnia pulex Simocephalus serrulatus	Acute toxicity	48-hr EC_{50} determined	(34)
Pesticides	Daphnia magna	Acute toxicity	LD_{50} values given for plant extracts	(50)
Carbonate insecticide	Daphnia magna	Determine toxicity of carbonate in water	LD_{50} determined	(33)
Herbicides	Six species of fresh-water crustacean including Daphnia magna	Acute toxicity	TL_{50} determined for 16 herbicides	(32)
Metals	Daphnia magna	Acute and chronic toxicity	Chronic toxicity of various metals given	(39)
NTA (Nitrilotriacetate) and Metal-NTA	Daphnia magna	Chronic toxicity	LC_{50} given	(51)
DDT	Daphnia magna	Report on accumulation of DDT by Daphnia	Daphnia do accumulate DDT stated need for further study	(35)

LIST OF SODIUM SALTS

Sodium acetate, S. asenate, S. arsenite, S. benzoate, S. borate, S. perborate, S. bromate, S. bromide, S. carbonate, S. bicarbonate, S. chlorate, S. chloride, S. dichromate, S. citrate, S. cyanide, S. ferrocyanide, S. fluoride, S. formate, S. hydroxide, S. iodade, S. idide, S. nitrate, S. nitrite, S. nitroprusside, S. oxalate, S. monolasic phosphate, S. dilasic phosphate, S. tribasic phosphate, S. salicylate, S. sulfate, S. bisulfate, S. sulfide, S. bisulfite, S. tartrate, S. throcyanate, S. thiosulfate.

CHLORIDES OF METALS USED

Sodium chloride, Calcium chloride, Magnesium chloride, Potassium chloride, Ammonium chloride, Strontium chloride, Stannic chloride, Manganese dichloride, Ferrous chloride, Lithium chloride, Antimony trichloride, Barium chloride, Stannous chloride, Ferric chloride, Aluminum chloride, Cobalt chloride, Chromic chloride, Nickel chloride, Lead chloride, Zinc chloride, Cupric chloride and Cupric ammonium chloride, Silver nitrate, Mururic chloride, Cadmium chloride.

HERBICIDES USED

Dichlone, molinate, Propanil, Sodium arsenite, Diquat, Dichlobenil, Paraquat, Amitrole, Amitrole T, Endothall, Diuron, Silvex fenac, Monuion, MCPA, 2, 4-D.

PESTICIDES USED

DDT, TDE(DDD), Methoxychlor, Toxaphene, Chlordane, Aldrin, Endrin, Heptachlor, Aramite, Dieldrin, Lindane, Chlorobenzilate DDVP, Parathion, Phosdrin, Dipterex, Daytex, Dibrom, Diazinon, Malathion, Ethyl guthion, Phosphamidon, Sevin, Zectran, Allethrin, Pyrethrins, Rotenone, Cryolite, Lime Sulfur, Trifuralin, Sodium arsenite, Diruron, Kuron, Paraquat, Esteron 99, Casoron, Fenac (Sodium salt), Dead X, IPC, Dalapon.

CARBAMATE USED

UC-10854, Aldicarb, UC-20047-5, UC-22463, RE-5353, RE-5655, Aminocarb, Propoxin Bay 37344, Hercules 5727, Hercules 7522H, Hercules 8717, Hercules 9699, PPC 3, Dimetilan, Pyramat, Drolan, Zectran, Carbaryl, MC-A-600.

METALS TESTED

Sodium, Calcium, Magnesium, Potassium, Iron, Manganese, Zinc, Cadmium, Strontium, Barium, Arsenic, Tin, Chromium, Aluminum, Gold, Nickel, Lead, Copper, Platinum, Cobalt, Mercury.

TAXONOMY

No review of the literature would be complete without discussing taxonomy. Surprisingly little has been added to our knowledge of cladoceran taxonomy and distribution since the work done by Birge (Reference 52). Any account of the cladocerans of the United States must rely heavily on this work. The taxonomic relationships of the various focuses of Daphnia pulex and Daphnia longispina is still a controversial matter. Depending chiefly on the shape of the head, Daphnia pulex exhibits a multitude of summer "species," "subspecies," "varieties," and "forms" in North America but not in Europe. Some of the more common variants, cyclomorphic and otherwise, have been designated as pulicaria, minnehaha, curviraostria, obtusa, retrocurva, and arcuata. Daphnia longispina, on the other hand, exhibits a comparable complex condition on both sides of the Atlantic. Common variants are hyalina, typica, mendotae, and galeata (Reference 2).

A few researchers like Mackin (Reference 49) take the opposite viewpoint. Mackin favors the concept that Daphnia longispina and pulex are two complex groups of species. Brooks (Reference 15), on the other hand, believes that Daphnia retrocurva is specifically distinct from Daphnia pulex and Daphnia galeata from Daphnia longispina on the basis of the appearance of a summer retrocurved helmet and spiked head, respectively, in some lakes. Daphnia galeata is commonly found in the eastern United States and is particularly common in New England.

No morphological criteria have yet been found by which the various forms of Daphnia longispina and Daphnia pulex complexes can be distinguished from each other during the colder months (October to March). During this season, all the individuals in each species constitute what is apparently a single morphological type, causing difficulty in attempting to establish numerous distinct species. A thorough taxonomic treatment of the Daphnia is being initiated with particular reference to the North American continent.

SECTION III

DISCUSSION

A great deal of research has been done using *Daphnia* as test animals. Most known information about feeding rates of aquatic invertebrates was first discovered about *Daphnia*. Toxicity studies first dealt with LIC_{50} and LC_{50}, but now researchers are looking at the sublethal, chronic effects of many industrial wastes. The problem of pesticide buildup and its effect is also getting more attention.

Since *Daphnia* are important to the aquatic food chain, any toxic material that affects their population could have catastrophic effects on high animals. It is important that more is known about the effect of accumulation of toxic matter in the aquatic ecosystem.

One of the problems that has generated considerable debate is cyclomorphosis (seasonal changes). Cyclomorphosis still remains a problem. There are several explanations for the changes that occur during the warmer temperature months.

Banta (Reference 16) indicates that temperature is by no means the whole story and that genetic factors probably play an important role.

The research using *Daphnia* has contributed greatly to our knowledge about the aquatic ecosystem. *Daphnia* have been used for years as test organisms and will continue to be used. The importance of this work can only be measured by our increasing knowledge of the impact of man on the aquatic ecosystem and ways to negate the impact.

SECTION IV

RECOMMENDATIONS

1. Continue research using Daphnia as a biological indicator of water quality.

2. The Environmental Health Laboratory, Kelly AFB, has a requirement for rapid and field expedient methodology for aquatic surveys using Daphnia. Field results must correlate with laboratory results.

3. Investigate the use of adenosine triphosphate (ATP) as a parameter in chronic toxicity studies.

4. Test whether sublethal amounts of JP-4 fuel, photographic wastes, fire fighter foam, and paint stripping wastes cause a change in the level of ATP in Daphnia. Determine whether this change can be correlated to environmental stress or used only to indicate the condition of a certain population of Daphnia.

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